Insulin's Structure as a Modified and Monomeric Molecule

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Insulin has a concentration of 10^{-8}–$10^{-11}M$ in the blood, which ensures that it circulates and brings about its biological effects as a monomer.[1] At higher concentrations, and in aqueous and neutral conditions, insulin dimerizes (Kd $\simeq 10^{-5}$), and in the presence of zinc ions, it further assembles to hexamers (Kd $\simeq 10^{-22}$), precluding the preparation of crystals containing native monomeric molecules.[1] It has been suggested[2] from the analysis of CD data that some unfolding of insulin's helices occurs at concentrations where it is a monomer. The crystal structure of a modified, active, and monomeric insulin has been determined by x-ray analysis; its structure remains very similar to that of the aggregated insulins and thus the hormone's assembly does not significantly alter its folding. Furthermore, the arguments relating insulin's biological action and structure, based on earlier crystal studies of the dimeric and hexameric molecule, remain valid.[1,3,4]

There are some exceptional insulins from the hystricomorph family that do not aggregate[1,3,5]; these, however, have not been crystallized and, in any event, have low potency and an altered structure. Our approach to these obstacles has been to crystallize an active modified insulin that does not aggregate. The modification is brought about by the enzymatic removal of the five B-chain C-terminal residues[6–8] that complete the H-bonding structure in the dimer and provide part of the nonpolar surface buried by dimer formation.[1] The potency of despentapeptide insulin (DPI) has been reported as 40% or higher[6–8]; our own material has a potency of about 40% (R. H. Jones and S. Tolley, unpublished results). Solution studies by Chinese workers have established that this modified molecule, DPI, does not aggregate.[8] Its general structural similarity to insulin is supported by spectroscopic studies and by the finding that its binding behavior with insulin antibodies is indistinguishable from that of the native hormone (Refs. 3, 8; and G. W. Reeves and T. C. Liang, personal communications).

The DPI is available from several species; the best crystals we have obtained were grown from beef and sheep material. The most detailed analysis, at 1.5-Å spacing, has been carried out on beef monoclinic (C2) DPI crystals; there is also an orthorhombic form.[9] The pig DPI analysis at 2.4-Å spacing was reported last July by the Peking group at Oxford's Biochemical Society meeting. This study is part of their systematic chemical, biological, and structural analyses of B-chain C-terminal residues. There are interesting differences between the crystal structures of pig and beef (and sheep) DPI, notably in the metal binding; the main conformational details appear, however, to be the same.

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The initial crystal structure of the beef (C2) DPI molecule was determined by molecular replacement procedures.[9] In these calculations, a model DPI molecule was suitably tailored from the known insulin structure in 2 Zn insulin by excluding B26–B30 and the structurally variable B-chain N-terminus. The agreement factor R ($R \equiv \Sigma|Fo| - |Fc|/\Sigma Fo$), for the observed structure factors Fo and those calculated from the model DPI, correctly positioned in the cell (Fc), was 0.52. (2.2-Å data). The atomic positions for much of this model were sufficiently correct for automatic refinement calculations to be used successfully. These have been carried out with 1.5-Å data (9750 terms) using the Agarwal fast-Fourier least-squares procedure to calculate atomic shifts and the MODELFIT program to impose the correct protein geometry.[10,11] During the refinement, 26 residues of the 90 in the asymmetric unit were repositioned in the electron density. The R factor is now 0.17; all the protein atoms have been located and 120 of the 180 water molecules have been identified. The well-defined atomic coordinates have an estimated error of about 0.1 Å; this increases to 0.3 Å for poorly defined atoms. Details of this refinement will be published elsewhere.

The two crystallographically independent DPI molecules and the two independent molecules in the 2 Zn insulin hexamer[12,13] are illustrated in Fig. 1. The most striking differences are, first, in the absence of the B30–B26 residues in DPI and the greatly altered structure at B1–B4. It is notable that the loss of the B-chain

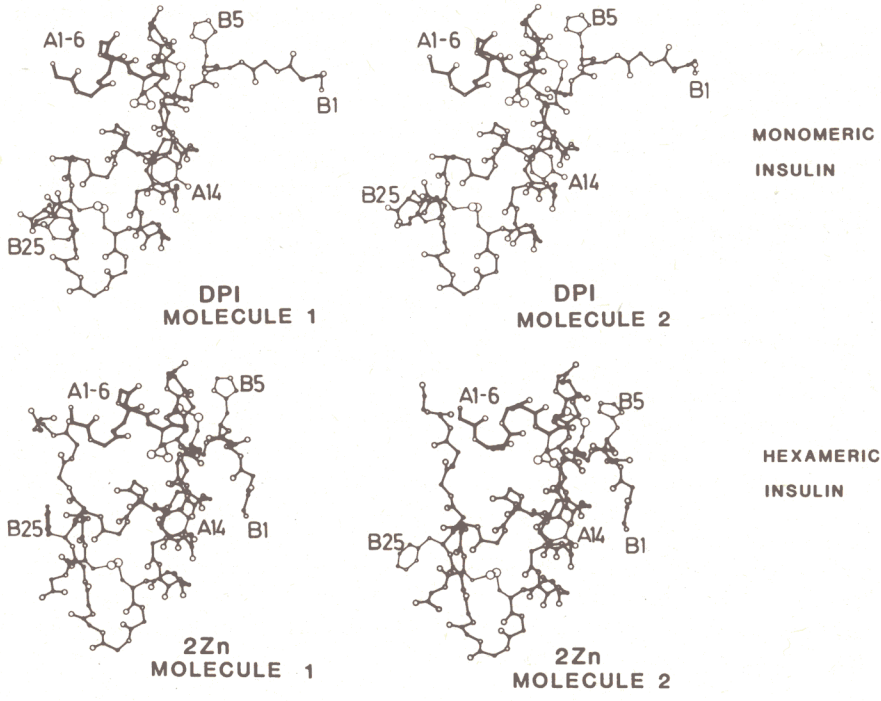

Fig. 1. The two 2 Zn insulin and DPI molecules viewed perpendicular to the normal dimer-forming surface. Only the main-chain atoms and selected side chains are shown. The direction of the residues B1–B4 in DPI away from the body of the molecule and the new conformation at B25 are clearly seen. The structures at A1–A6 in the two 2 Zn insulin molecules are different. In DPI, these residues have the same conformation found in 2 Zn insulin molecule 1.

C-terminal residues, although largely unshielding the A-chain and removing a number of H-bonding and nonpolar contacts, does not affect the A-chain and its adjacent B-chain structure significantly. There is, by contrast, an important change in structure at B25 Phe, in DPI, the B-chain C-terminus. Evidently the removal of B26–B30 has released B25 Phe from both its H-bonding contact (B25NH to Al9CO) and its side-chain nonpolar contacts to A19.

Second, the residues B1–B4 in DPI assume a new conformation in which they are directed away from the molecule. This arrangement breaks the pair of anti-parallel B-sheet H-bonds between B4 and A11 which are now H-bonded to water (Fig. 2), increasing the exposure of the A-chain to solvent. The variation in structure at B1–B4 is also seen in 4 Zn insulin, where it is helical,[14] and in hagfish insulin, where, as in DPI, it is directed away from the A-chain (C. D. Reynolds, manuscript in preparation).

The RMS difference between the insulin and DPI molecules for the polypeptide backbone atoms of the A-chain and the B-chain, for residues B8–B23, is listed in Table I. The similarity in the structure of the A-chain and the adjacent B-chain residues in insulin and DPI, illustrated in Fig. 3, establishes that B30–B26 and B4–B1 are not critical in defining the hormone's folding, and confirms the reversible character of the H-bonding between B4 and B25 to the A-chain (Ref. 14; and Reynolds, manuscript in preparation). The small changes in the DPI and insulin structures largely result from the small relative movements made by the more rigid helical segments. It is through movements of this kind, described recently for 2 and 4 Zn insulin, that the different aggregation and packing forces are accommodated by the molecule.[15] We conclude from the above that the assembly of the monomer to the dimer and to the 2 Zn insulin hexamer does not alter its structure in any important way. We also conclude that the monomer has a definite 3-dimensional structure in solution identical in its essentials to insulin in the 2 Zn insulin hexamer (see Fig. 1). There is a distinctly closer correspondence between DPI and the 2 Zn insulin molecule 1, particularly noticeable between A1 and A6, which confirms the proposal that it is nearest the structure of the free monomer.[13] In

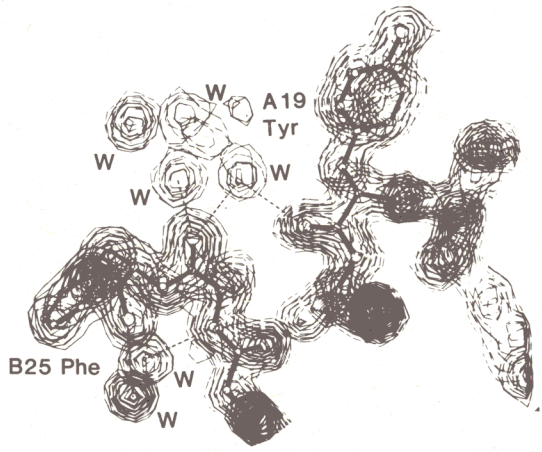

Fig. 2. The electron density at A19 tyrosine and B24 and B25 phenylalanine is the DPI 1.5-Å spacing electron density map calculated with coefficients $\|2Fo| - |Fc\|$. This illustrates how the B25 residue is turned away from the A-chain in DPI and how the water molecules (labelled W) have provided the H-bonding interactions to these residues which are exposed in DPI.

TABLE I
RMS Differences between 2 Zn Insulin and DPI Molecule's Main-Chain Structure[a]

	2 Zn Insulin Molecule 1	2 Zn Insulin Molecule 2	DPI Molecule 1	DPI Molecule 2
2 Zn insulin, molecule 1		0.7 Å	0.6 Å	0.8 Å
2 Zn insulin, molecule 2	0.7 Å		1.0 Å	1.1 Å
DPI, molecule 1	0.6 Å	1.0 Å		0.5 Å
DPI, molecule 2	0.8 Å	1.1 Å	0.5 Å	

[a] Comparisons are between the polypeptide backbone of the residues A1–A20 and B5–B23. The best match is between the two DPI molecules and between DPI molecule 1 and 2 Zn insulin molecule 1. The poorer agreement between 2 Zn insulin molecule 2 and the other molecules is a consequence of the rearrangement of the A1–A6 residues in the R_3 crystal. There is considerable evidence that the 2 Zn insulin molecule 1 is nearest the free monomer structure (Ref. 13).

this paper, we have adopted the Chinese convention for numbering the two independent molecules in the 2 Zn insulin asymmetric unit. (See Ref. 13 for details.)

Therefore, the structural framework in which the many chemical sequence changes in insulin are related to its biological behavior proves to be valid. The evidence that the monomer unfolds to some extent in solution[2] appears to be challenged by the DPI crystal structure; the significance of the observed changes in the insulin's CD spectrum at low concentrations needs to be investigated. Finally, the substantial activity of DPI can be understood in terms of the preserved surface structure in the A-chain and central B-chain residues. This reinforces the proposal, based on recent biological and structural studies on modified insulins,[16–19] that the contacts directly responsible for insulin action are made through the surface structure on the A-chain and adjacent B-chain residues, and not through the dimer-forming region. It also seems probable that the carbonyl oxygens at the C-terminus of the A-chain and the peptide groups of the surrounding B-chain residues, which in DPI remain exposed and H-bonded to water, participate in the insulin-receptor interaction.[19]

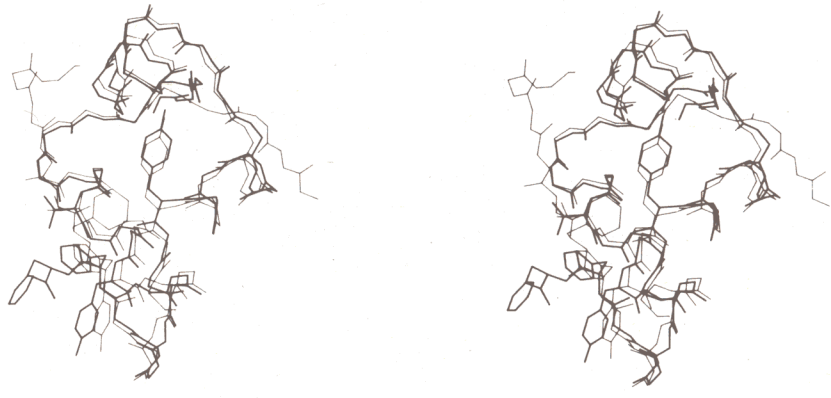

Fig. 3. A stereo figure of the 2 Zn insulin molecule (thin lines) overlapped onto DPI molecule 1 (thick lines). The molecules are viewed in the direction of the local twofold axis present in 2 Zn insulin. The structure at B1–B4 and at B25 in DPI has broken the H-bond contacts present in 2 Zn insulin.

The structural variation exhibited by DPI in the beef DPI crystals is reminiscent of that found with insulin in the 2 Zn insulin crystal. The capacity of DPI to vary its structure as substantially as does insulin in 4 Zn insulin has not yet been detected.[16,20] This possible capacity and its role in the expression of insulin's activity will be the subject of future research.

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